Hatchery workers' IgG antibody profiles to airborne bacteria

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ABSTRACT

Occupational exposure to high concentrations of airborne bacteria in poultry production is related to an increased risk of respiratory disorders. However, etiology and in particular microorganisms' potential role in pathogenesis still needs to be elucidated. Thus, detection of specific antibodies against occupational microbial antigens may lead to identification of potentially harmful species. For the purpose of IgG titer determination, indirect immunofluorescence on various bacterial isolates from duck hatchery air was combined with image-based quantification of fluorescence intensity. Moreover, in addition to established assays with pure bacterial cultures, a new approach utilized complex bioaerosol samples for detection of anti-microbial antibodies in human sera by determination of percentages of antibody-bound cells in different serum dilutions. Mean titers in sera from hatchery workers and a non-exposed control group did not display significant differences for most tested isolates and application of comprehensive cluster analysis to entire titer data revealed no structure reflecting workers and controls group. Furthermore, determination of immunoreactivity to the complete microbial community in workplace air displayed similar proportions of antibody-bound cells in both groups. Although no general differences in immunoreaction patterns were observed, mean titers to a *Proteus mirabilis* isolate and to 3 of 4 distinct *Acinetobacter baumannii* isolates were higher in the group of hatchery workers than in the reference group indicating a potential applicability as exposure markers. We conclude, despite long term bioaerosol exposure, hatchery workers' IgG antibody profiles to tested antigens did not differ substantially from those of the control group. However, increased workers' titers to *A. baumannii* and clinical relevance of this species should lead to further investigations regarding potential involvement in pathogenesis of occupational respiratory disorders.

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1. Introduction

Current intensive animal husbandry with high stocking densities leads to emergence of workplaces that are rarely characterized in regard to microbial exposure. Long term inhalation of agricultural bioaerosols may cause a wide range of adverse health effects, particularly a decline in lung function, chronic obstructive pulmonary disease and hypersensitivity pneumonitis (Donham et al., 2000; Lacey and Dutkiewicz, 1994; Lenhart and Olenchock, 1984; Martin et al., 2013; Radon et al., 2001; Rylander and Carvalheiro, 2006). However, causalities between occupational respiratory disorders and inhalation of complex bioaerosols are not well understood. Since these diseases often share immunological features, serological approaches utilizing specific antigen-antibody reactions enable detection of specific immunoglobulins (IgE, IgG)

against work-related microbial antigens and may help to comprehend etiology. However, attention should be paid to the different inflammatory mechanisms following either infection or sensitization. Allergic disorders are pathophysiologic immunoreactions marked by an immune-specific inflammation including a major role of different antibody classes. While allergic rhinitis and asthma are associated with IgE mediated sensitization (type I allergy), hypersensitivity pneumonitis (extrinsic allergic alveolitis/farmers' lung) is associated to IgG (type III allergy) (Douwes et al., 2003; Fung and Hughson, 2003). Antibody responses to infections typically arise within 10–14 days of initial exposure (first IgM, later IgG) and can persist over years or decades, thus reflecting an individual history of pathogen encounters. For diagnostics of sensitizations to inhalable allergens in IgE-mediated allergic diseases skin prick or skin patch tests are essential procedures (Rees et al., 1998; Rimac et al., 2010; Zuskin et al., 1994). Currently, sensitive detection and precise quantification of immunoglobulins is performed by using enzyme-linked immunosorbent assays (ELISA) (Luttmann et al., 2014). This approach enables analysis of seroprevalences of different antibody

classes either involved in allergic diseases or infections, for example determination of specific anti-bacterial or anti-fungal IgG (Bünger et al., 2000; Laitinen et al., 1999; Tillie-Leblond et al., 2011), specific anti-bacterial or anti-fungal IgE (Doekes et al., 2004; Scalabrin et al., 1999) or total IgE (Rimac et al., 2010; Skorska et al., 2007). Moreover, also commercial systems applying ELISA technology with standardized antigens for antibody quantification are available (e.g. ImmunoCAP). Especially for serological diagnosis of infections a combination of techniques might be useful, e.g. ELISA for screening of sera and western blot as confirmation test (Porsch-Özcürümez et al., 2004). Nevertheless, establishment of an individual ELISA protocol for a single test antigen is time-consuming and it is even more when antibody titers to numerous isolates are intended to be determined. Compared to other test principles, implementation of indirect immunofluorescence (IIF) testing is convenient when using whole-cell antigens, so this technique allows simple and rapid analysis of specific antibody seroprevalences.

In clinical routine serology indirect immunofluorescence is applied for serodiagnosis of infections from *Bartonella henselae*, *Legionella pneumophila*, *Francisella tularensis* and *Chlamydia* sp. (Amerein et al., 1996; Baud et al., 2010; Benson et al., 1983; Jenzora et al., 2008; Wilkinson et al., 1979). However, commercial indirect immunofluorescence systems often have been developed for specific antibody detection of aforementioned well-known clinical pathogens and are in most cases not available for the diverse species identified in animal husbandry. Furthermore, their limited applicability for field studies may lead to imprecise detection due to the diversity of antigenic properties observed in large numbers of different occupational bacterial species and strains.

Previous investigations in a duck hatchery revealed occurrence of high concentrations of airborne bacteria (up to 2×10^7 cells m^{-3} and 7×10^6 CFU m^{-3}) during sorting of ducklings and a dominance of the genera *Staphylococcus*, *Enterococcus* and *Acinetobacter* (Martin and Jäckel, 2011). Furthermore, workers exhibited a decline in lung function over their work shifts at days when freshly hatched ducklings were removed from incubators (Martin et al., 2013). The present study aimed at characterization of serological profiles from workers of the same duck hatchery. In order to assess the immunogenic potential of workplace species individual titers to eleven isolates from duck hatchery bioaerosols were determined using dedicated *Fluorolyzer* software for automated image analysis. This approach avoids major drawbacks of conventional titer determination by eye like significant inter-observer variability and difficult interpretation of borderline samples (Bakken et al., 1992; Chiaro et al., 2011; Luger and Krauss, 1990; Peeling et al., 2000; Wilkinson et al., 2003). Furthermore, this study presents a new approach in anti-microbial antibody detection by employing complex bioaerosol samples as antigens for indirect immunofluorescence testing.

2. Material and methods

2.1. Study subjects

Sera were collected from workers (n = 10) employed in a German duck hatchery and a non-exposed reference group (n = 10) in 2010 (kindly provided by G. Linsel, Berlin). The reference group was recruited from employees of the Federal Institute for Occupational Safety and Health (Berlin). Both groups comprised each 3 females and 7 males. The age distribution in the worker group was 23–57 years (mean 38.9, sd 11.3) and in the control group 34–58 years (mean 49.5, sd 7.6). The duration of employment in the investigated hatchery was 5–308 months (mean 72.6, sd 87.7). Typical work activities at those days included manual removal of hatcher baskets containing emerged ducklings from incubators and manual sorting for separation of healthy ducklings from eggshells and dead or inadequate animals. Furthermore, cleaning of equipment, rooms and incubators was performed with high-pressure cleaners. At days without regular removal of ducklings concentrations of airborne bacteria were lower by several orders of magnitude. Typical tasks at those days included candling of eggs for monitoring the development of embryos, manual transfer of eggs from setter incubators to hatcher incubators as well as general maintenance.

2.2. Bacterial isolates

A total of eleven duck hatchery bacterial isolates were employed for antibody detection (kindly provided by E. Martin, Berlin) (Martin and Jäckel, 2011): I B-1, I B-2, I B-3, II B-9 (all *Acinetobacter baumannii*), I B-4 (*Enterococcus faecium*), I B-5 (*Staphylococcus aureus*), I B-6 (*Staphylococcus delphini*), I B-7 (*Chryseobacterium aquifrigidense*), I B-15 (*Pusillimonas* sp.), II B-13 (*E. coli*), II B-18 (*Proteus mirabilis*). Isolates were recovered from workplace by collecting bioaersols on gelatin filters and were identified by comparison of 16S rRNA gene sequences with provided sequences of bacterial type strains in GenBank. For indirect immunofluorescence assays isolates were grown on casein-soy agar (Carl Roth, Germany) for 24–48 h. Bacterial colonies were harvested and suspended in 0.9% NaCl and subsequently inactivated and fixed by the addition of ethanol to a final concentration of 50% (vol/vol).

2.3. Strain typing of Acinetobacter baumannii isolates

Bacterial strain typing of four *A. baumannii* isolates (I B-1, I B-2, I B-3, II B-9) was performed by ApaI-macrorestriction followed by pulsed-field gel electrophoresis (PFGE) with interpretation of results according to the criteria of Tenover et al. (1995). Further typing included assignment to the important international clones 1–3 (formerly named European clones I–III) by multiplex-PCR and *bla*$_{OXA-51-like}$ gene sequencing (Turton et al., 2007; Zander et al., 2012). Classification to the species *A. baumannii* was determined by near full length sequencing of the 16S rRNA gene (Weisburg et al., 1991) and partial sequencing of the *rpo*B gene (Nemec et al., 2009).

2.4. Bioaerosol sampling

Bioaerosol samples were collected on polycarbonate filters (Ø 76 mm, 0.8 μm pore size; Whatman, Germany) in a duck hatchery during sorting of ducklings for 3 h with a flow rate of 1.8 m^3 h^{-1} using filtration devices (MD8 aluminum stacks; Sartorius, Germany; MP2/39 pump, Holbach GmbH, Germany). Microbial cells were detached from filter surface into 10 ml 0.9% NaCl using a Stomacher (Stomacher 80 Biomaster; Seward, UK). Three parallel samples were pooled and stabilized by the addition of 10% of a 37% formaldehyde solution.

2.5. Indirect immunofluorescence assays

Indirect immunofluorescence assays (IIFA) were performed according to a modified protocol described originally by Jenzora et al. (2008). IIFA were applied to cells from bacterial isolates and to microbial cells from bioaerosol samples. Cavities of diagnostic slides were loaded with 4×10^5 cells and air-dried. After incubation with blocking agent (5% goat serum (Sigma-Aldrich, USA) and 1% bovine serum albumin (Carl Roth, Germany) in PBS) for 30 min at room temperature, sera were added in two-fold serial dilutions (11 dilutions + no serum control (NSC)) and incubated for 60 min at 37 °C. After washing, Alexa Fluor 488® conjugated goat anti-human IgG secondary antibody was added for 30 min at 37 °C. Bacterial DNA was stained with 4′,6-diamidino-2-phenylindole (DAPI). After